Published in "Microbial Drug Resistance doi: 10.1089/mdr.2018.0153, 2018"
which should be cited to refer to this work.

Performances of the Rapid Polymyxin *Acinetobacter* and *Pseudomonas* Tests for Colistin Susceptibility Testing

Mathilde Lescat,[1–4] Laurent Poirel,[1,2,5] Aurélie Jayol,[1,2,5] and Patrice Nordmann[1,2,5,6]

Objectives: Owing to the emergence of colistin resistance in nonfermenting Gram negative bacteria, reliable and rapid techniques for testing colistin susceptibility are needed. We evaluated the performances of the Rapid Polymyxin *Acinetobacter* and *Pseudomonas* tests using a collection of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* clinical isolates.
Methods: Colistin susceptibility of *A. baumannii* and *P. aeruginosa* isolates (colistin susceptible and colistin resistant) was tested with the Rapid Polymyxin *Acinetobacter* and *Pseudomonas* tests and compared with the broth microdilution method.
Results: The Rapid Polymyxin *Acinetobacter* and *Pseudomonas* tests were able to detect all colistin-resistant and all colistin-susceptible *A. baumannii* and *P. aeruginosa* isolates within 4 hours.
Conclusion: The Rapid Polymyxin *Acinetobacter* and *Pseudomonas* tests are reliable techniques for detecting colistin resistance. Overall, both techniques allow an accurate and a rapid screening (<4 hours) of colistin resistance in *A. baumannii* and *P. aeruginosa*.

Keywords: susceptibility testing, rapid diagnostic test, polymyxin, *Acinetobacter*, *Pseudomonas*

Introduction

Acinetobacter baumannii and *Pseudomonas aeruginosa* belong to the ESKAPE group of pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) identified as the most important bacterial species in clinical settings as a source of multidrug resistance.[1] Infections due to multidrug-resistant (MDR) *A. baumannii* and *P. aeruginosa* species, especially carbapenem-resistant isolates, are increasingly reported in health care facilities, being responsible for nosocomial infections that may lead to fatal outcomes due to limited therapeutic options.[2–4] The Center for Diseases Control of Atlanta in the United States and then the World Health Organization classified the carbapenem-resistant *A. baumannii* and *P. aeruginosa* among the most serious pathogens exhibiting multidrug resistance.[5,6] Consequently, old antibiotics such as polymyxins (colistin) are increasingly used as last resort treatment for treating MDR *A. baumannii* and *P. aeruginosa.*[4,7] This highlights the importance of giving rapid results of polymyxin susceptibility to optimize the antibiotic stewardship.

The current standard method of detection for colistin susceptibility in Gram negatives is the manual determination of minimum inhibitory concentration (MIC) by the broth microdilution (BMD) method.[8] However, this procedure is technician dependent (partly due to the fact that colistin must be weighted for each experiment), is time consuming, and results are obtained in 24 hours.

Recently, Nordmann *et al.* developed the Rapid Polymyxin Nordmann-Poirel (NP) test that categorizes colistin-susceptible from colistin-resistant enterobacterial isolates in <2 hours.[9] However, this test based on visualization of glucose metabolization cannot be applied to nonfermenting Gram negative bacteria, such as *A. baumannii* and *P. aeruginosa.*

Using the same principle as the Rapid Polymyxin NP test, the Rapid Polymyxin *Acinetobacter* and *Pseudomonas* tests have recently been developed by Elitech Microbiology (www.elitechgroup.com/france) and are liquid-based techniques that rely on the colorimetric detection of a rapid metabolism related to bacterial growth, in the presence of a defined concentration of colistin. The acidification of the medium related to bacterial growth is visualized by the color shift of the pH indicator (red to yellow or orange with the red phenol in the Rapid Polymyxin *Acinetobacter* test and

[1]Emerging Antibiotic Resistance Unit, Medical and Molecular Microbiology, Department of Medicine, Faculty of Science and Medicine, University of Fribourg, Fribourg, Switzerland.
[2]INSERM European Unit (LEA, IAME), Paris France.
[3]Université Paris Nord, Sorbonne Paris Cité, Paris, France.
[4]AP-HP, Hôpitaux Universitaires Paris Seine Saint-Denis, Paris, France.
[5]Swiss National Reference Center for Emerging Antibiotic Resistance (NARA), University of Fribourg, Fribourg, Switzerland.
[6]Institute of Microbiology, University of Lausanne and University Hospital Center, Lausanne, Switzerland.

green to violet with bromocresol purple in the Rapid Polymyxin *Pseudomonas* test).

The objective of this study was to evaluate the performance of those novel tests by comparison with the BMD method using a collection of colistin-susceptible and colistin-resistant *A. baumannii* and *P. aeruginosa* clinical isolates.

Materials and Methods

Bacterial strains

This study was carried out using 38 clinical isolates of *A. baumannii* ($n = 21$) and *P. aeruginosa* ($n = 17$) identified at the species level using the Microflex benchtop matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometer (Brücker, Champs-sur-Marne, France). Nine out of the 21 *A. baumannii* isolates and 10 out of the 17 *P. aeruginosa* isolates were susceptible to colistin. Twelve out of the 21 *A. baumannii* and 7 out of the 17 *P. aeruginosa* isolates were colistin resistant according to BMD testing. Isolates were grown on Luria Bertani (LB; Sigma, Saint Louis, MO) agar plates at 35°C ± 2°C for 18 hours. The colistin-susceptible strain *P. aeruginosa* ATCC 27853 and the colistin-resistant *Escherichia coli* R2739 were used as negative and positive controls, respectively, for the determination of MIC of colistin by the BMD method. None of the colistin-resistant isolates carried a plasmid-encoded MCR-like (MCR-1 to -4) colistin resistance determinant, as assessed by the negative polymerase chain reaction results (data not shown).

Susceptibility testing

The BMD method was performed in triplicate and interpreted according to the EUCAST/CLSI joined guidelines[8] as described.[10]

Table 1. Minimum Inhibitory Concentrations of Colistin (mg/L) Using the Broth Microdilution Method and Results of the Rapid Polymyxin *Acinetobacter* and *Pseudomonas* Tests

					Rapid Polymyxin Acinetobacter/ Pseudomonas *test*	
Isolate	*Species*	*Origin*	*Phenotype*	*BMD MIC colistin (mg/L)*	*MIC colistin (mg/L)*	*Discrepancies*[a]
FR-242	*Acinetobacter baumannii*	Switzerland	S	<0.12	≤2	No
FR-243	*A. baumannii*	Turkey	S	<0.12	≤2	No
FR-244	*A. baumannii*	Turkey	S	<0.12	≤2	No
FR-245	*A. baumannii*	Turkey	S	<0.12	≤2	No
FR-246	*A. baumannii*	Turkey	S	<0.12	≤2	No
FR-247	*A. baumannii*	Turkey	S	<0.12	≤2	No
FR-248	*A. baumannii*	Turkey	S	<0.12	≤2	No
N4	*A. baumannii*	Switzerland	S	0.25	≤2	No
N14	*A. baumannii*	Switzerland	S	<0.12	≤2	No
FR-250	*A. baumannii*	Italy	R	8	>4	No
FR-252	*A. baumannii*	Italy	R	64	>4	No
FR-253	*A. baumannii*	Spain	R	4	>4	No
FR-254	*A. baumannii*	Spain	R	16	>4	No
FR-255	*A. baumannii*	Switzerland	R	128	>4	No
FR-256	*A. baumannii*	Turkey	R	16	>4	No
FR-257	*A. baumannii*	Turkey	R	8	>4	No
FR-258	*A. baumannii*	Turkey	R	4	>4	No
FR-259	*A. baumannii*	Turkey	R	4	>4	No
FR-260	*A. baumannii*	Turkey	R	>128	>4	No
FR-261	*A. baumannii*	Turkey	R	4	>4	No
FR-262	*A. baumannii*	Turkey	R	>128	>4	No
ATCC 27853	*Pseudomonas aeruginosa*	United States	S	<0.12	≤2	No
FR-263	*P. aeruginosa*	France	S	<0.12	≤2	No
FR-264	*P. aeruginosa*	France	S	<0.12	≤2	No
FR-265	*P. aeruginosa*	France	S	<0.12	≤2	No
FR-266	*P. aeruginosa*	France	S	<0.12	≤2	No
FR-267	*P. aeruginosa*	France	S	<0.12	≤2	No
FR-268	*P. aeruginosa*	France	S	<0.12	≤2	No
FR-269	*P. aeruginosa*	France	S	<0.12	≤2	No
FR-270	*P. aeruginosa*	France	S	0.25	≤2	No
FR-271	*P. aeruginosa*	France	S	0.25	≤2	No
FR-274	*P. aeruginosa*	France	R	4	8	No
FR-275	*P. aeruginosa*	France	R	32	8	No
FR-276	*P. aeruginosa*	France	R	32	>8	No
FR-277	*P. aeruginosa*	France	R	16	8	No
FR-278	*P. aeruginosa*	France	R	128	>8	No
FR-279	*P. aeruginosa*	France	R	8	4	No
FR-281	*P. aeruginosa*	France	R	4	>8	No

The colistin-resistant isolates are shaded in gray.
BMD, broth microdilution; MIC, minimum inhibitory concentration; R, resistant; S, susceptible.

Rapid Polymyxin Acinetobacter *and* Pseudomonas *tests*

The Rapid Polymyxin *Acinetobacter* and *Pseudomonas* tests from Elitech Microbiology (www.elitechgroup.com/france) were performed according the manufacturer instructions. In brief, a standardized suspension of each isolate is prepared using a medium specific to each species. A specific volume of this suspension is then placed in the different wells of the kit containing defined amounts of colistin to obtain final concentrations of 0 (positive control), 2, and 4 mg/L for both species, and in addition of 8 mg/L for *P. aeruginosa*. A supplementary well is used as a negative control in which a suspension with only NaCl is prepared. After 3–4 hours of incubation at 37°C, a valid result is obtained when a color shift is observed in the positive control well, and no color shift in the negative control well. The result is then read for each colistin containing well as an MIC reading. For this evaluation, the results of susceptibility/resistance to polymyxins for each isolate were observed after 2 hours every 15 minutes until 4 hours.

Result analysis

The results obtained with the Rapid Polymyxin tests were compared with those obtained with the reference BMD method. In brief, discrepancies were determined to assess their performance to detect colistin susceptibility. Very major errors (VME) and major errors (ME) corresponding to false-susceptible and false-resistant results, respectively, were calculated as described elsewhere.[11,12]

Results

A total of 21 *A. baumannii* and 17 *P. aeruginosa* isolates were included to evaluate the performances of the Rapid Polymyxin *Acinetobacter* and *Pseudomonas* tests (Table 1). All of the nine *A. baumannii* isolates defined as colistin susceptible according to the result of the BMD method (MICs of colistin ranging from <0.125 to 0.25 mg/L) were identified as susceptible by the Rapid Polymyxin *Acinetobacter* test (Table 1). All of the 12 colistin-resistant *A. baumannii* isolates (MICs of colistin ranging from 4 to >128 mg/L) were detected as colistin resistant by the Rapid Polymyxin *Acinetobacter* test (Table 1). Out of the 10 colistin-susceptible *P. aeruginosa* isolates (MICs of colistin ranging from <0.125 to 0.25 mg/L), all were found susceptible using the Rapid Polymyxin *Pseudomonas* test. All of the seven colistin-resistant *P. aeruginosa* isolates (MICs of colistin ranging from 4 to 128 mg/L) were identified as colistin resistant by the Rapid Polymyxin *Pseudomonas* test (Table 1). Interpretation of the results for all isolates was obtained between 3 and 4 hours; no positive result was observed before 3 hours.

Discussion

Out of the 19 colistin-susceptible *A. baumannii and P. aeruginosa* isolates, the Rapid Polymyxin *Acinetobacter* test identified correctly all susceptible isolates, hence no ME (*i.e.*, false resistance) was detected for both tests (specificities of 100%). Out of the 19 colistin-resistant *A. baumannii* and *P. aeruginosa* isolates, the Rapid Polymyxin *Acinetobacter* and *Pseudomonas* tests were excellent with no VME (*i.e.*, false susceptibility; sensitivities of 100%).

This study showed that the Rapid Polymyxin *Acinetobacter* and *Pseudomonas* tests are reliable tools for detecting resistance to colistin in *A. baumannii* and *P. aeruginosa.* In comparison, BMD systems (Sensititre [ThermoFischer Diagnostics] and UMIC [Biocentric and MicroScan]) have been evaluated and showed VME for the three systems and ME for the MicroScan system.[13] Moreover, these are the first tests that are available for determination of colistin susceptibility in those species in <4 hours. However, other evaluations with a higher number of isolates should be performed to confirm our results as well as at least a multicenter study and its possible interest directly from clinical samples. Although MICs of colistin are only determined in ranges (≤2, comprised between 2 and 4 mg/L, and >4 mg/L for *A. baumannii* and ≤2, comprised between 4 and 8 mg/L, and >8 mg/L for *P. aeruginosa*) using those tests, they give results of susceptibility/resistance categorization very rapidly, which is the most important feature with respect to the treatment strategy and may contribute to optimize antibiotic stewardship. Colistin is indeed often used to treat infections caused by MDR *A. baumannii* and *P. aeruginosa* isolates, mostly remaining susceptible to colistin.[14] However, the increasing trend of acquired colistin resistance observed in those species, and particularly in *A. baumannii*,[15] highlights the interest to use such rapid tests able to efficiently detect that resistance trait.

Acknowledgments

We thank the Elitech Microbiology company for providing us kits of Rapid Polymyxin *Acinetobacter* and *Pseudomonas* tests. We thank Javier Fernandez, Véronique Dubois, and Deniz Güneşer for providing several resistant strains. This work has been funded by the University of Fribourg and by the Swiss National Reference Center for Emerging Antibiotic Resistance, Fribourg, Switzerland. It has also been funded by the Swiss National Science Foundation (projects FNS-407240_177381 and FNS-407240_177382).

Disclosure Statement

No competing financial interests exist.

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Address correspondence to:
Laurent Poirel, PhD
Emerging Antibiotic Resistance Unit
Medical and Molecular Microbiology
Department of Medicine
Faculty of Science and Medicine
University of Fribourg
Chemin du Musée 18
CH-1700 Fribourg
Switzerland

E-mail: laurent.poirel@unifr.ch